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Characterization of Rat Cecum Cellulolytic Bacteria

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Cellulose-degrading bacteria previously isolated from the ceca of rats have been characterized and identified. The most commonly isolated type was rods identified as *Bacteroides succinogenes*. These bacteria fermented only cellulose (e.g., pebble-milled Whatman no. 1 filter paper), cellobiose, and in 43 of 47 strains, glucose, with succinic and acetic acids as the major products. The only organic growth factors found to be required by selected strains were *p*-aminobenzoic acid, cyanocobalamine, thiamine, and a straight-chain and a branched-chain volatile fatty acid. These vitamin requirements differ from those of rumen strains of *B. succinogenes*, indicating the rat strains may form a distinct subgroup within the species. The mole percent guanine plus cytosine was 45%, a value lower than those (48 to 51%) found for three rumen strains of *B. succinogenes* included in this study. Cellulolytic cocci were isolated less frequently than the rods and were identified as *Rumminococcus flavefaciens*. Most strains fermented only cellulose and cellobiose, and their major fermentation products were also succinic and acetic acids. Their required growth factors were not identified but were supplied by rumen fluid.

Although the cellulolytic bacteria of the rumen have been the subject of much study (e.g., 1, 4, 5, 26), less attention has been paid to cellulolytic bacteria of the monogastric gut. This limited work includes the isolation of strains of *Ruminococcus flavefaciens* from the ceca of rabbits (22) and a guinea pig (19), as well as isolation of five types of cellulolytic bacteria from the large intestine of horses; these five were not identified, but two resembled *Bacteroides* spp. (16). A cellulolytic *Bacteroides* sp. has also been isolated from human feces (3), and cellulolytic bacteria isolated in low numbers from human feces (ca. 10^2/g; unpublished data) have been presumptively identified as *Ruminococcus* sp. and *Eubacterium cellulosolvens*.

As part of an investigation of the microflora of the rat gut (34), cellulolytic bacteria from the cecum were enumerated and selectively isolated. The two types isolated resembled previously described rumen cellulolytic strains of *Bacteroides succinogenes* and *R. flavefaciens*. In this paper, we describe the characterization of members of the two groups, concentrating primarily on the *B. succinogenes* strains, which were the predominant culturable cellulolytic organisms of the rat cecum. The information gained will be useful in further studies concerning the digestion of fiber by the *B. succinogenes* strains. For comparison, three rumen isolates of *Bacteroides succinogenes* were included in some of the characterization experiments: S85, the neotype strain (10), A3c (17), and REH9-1, chosen for its morphological similarity to the original isolates of the species (24, 25).

The cellulolytic isolates from rats were determined to be members of the species *B. succinogenes* and *R. flavefaciens*. The results reported here suggest that the species *B. succinogenes* comprises two or more subgroups distinguished from each other by vitamin requirements and DNA base composition.

MATERIALS AND METHODS

Organisms. The isolation of cellulolytic bacteria from rat cecal contents is described in the accompanying paper (34). The strains selected for the present study were BCO1 (a glucose nonfermenter) and BCX7 and NR9 (glucose fermenters). *B. succinogenes* S85 and A3c were obtained from M. P. Bryant; a culture of S85 was also obtained from C. W. Forsberg. *B. succinogenes* REH9-1 was isolated by R. E. Hungate from the rumen of an alfalfa-fed heifer. *Escherichia coli* K-12 was obtained from M. Miranda.

Culture techniques and media. The anaerobic culture techniques of Hungate (27) were used; cultures were incubated at 38°C. PY-CF agar and broth have been described elsewhere (34). The basic mineral medium, prepared under a CO_2 atmosphere, contained (per liter): 167 ml each of mineral solutions A and B (34), 5.0 g of $NaHCO_3$, 0.25 g of cysteine · HCl · H_2O, and 0.25 g of Na_2S · $9H_2O$. Where indicated in Results, the concentrations of solutions A and B were doubled (2XAB medium). Pebble-milled cellulose (PMC) media included the components of the basic mineral medium and 0.67% (dry wt/vol) PMC (PMC broth) or 0.5% PMC and 0.5% (wt/vol) purified agar (PMC agar).

Vitamin (40) and volatile fatty acid (VFA) (34) mixtures were also present in the PMC media, except where specific components were omitted for nutritional experiments or determination of products. These supplements could be added either before autoclaving or after separate sterilization. In experiments with the rod-shaped isolates, no difference in growth was detected when the vitamins were sterilized by filtration instead of autoclaving.

Substrates. The range of fermentable substrates was determined in PY-CF broth; turbidity and, in some cases, final pH were compared to appropriate controls. Fermentation of insoluble substrates was detected by disappearance of the substrates, e.g., up to an estimated 95% decrease in the settled volume of PMC.

PMC was prepared by pebble milling 20 g of Whatman no. 1 filter paper in 1 liter of distilled water for 16 h at 4°C. Pebble-milled spinach was prepared as follows. Leaves and stems of New Zealand spinach were chopped in a blender with sufficient water to make a thick slurry. The solids were repeatedly extracted at room temperature with water, 95% ethanol, and water again and then pebble milled as above. Hemicellulose was prepared by the method of Clarke (12). The insoluble substrates were added to the medium before autoclaving.

Product determination. Hydrogen was analyzed at room temperature on a Perkin-Elmer 154B gas chromatograph with a silica gel column and N_2 as the carrier gas. Formic acid was analyzed by the method of Lang and Lang (31). All other VFA were measured with a Varian model 3700 gas chromatograph equipped with a flame ionization detector under the following conditions: stainless steel column (6 ft- by 0.093-in. inner diameter [1.85 m by 2.4 mm]) packed with 15% FFAP chromosan acid wash; injector, 200°C; oven, 144°C; detector, 219°C; carrier gas, argon at 16 psig. D- and L-lactic (36) and succinic (35) acids were estimated enzymatically.

Scanning electron microscopy. Cultures were grown overnight in 2XAB cellobiose broth or PMC broth; the medium for the rumen strains was supplemented with 20% clarified rumen fluid (autoclaved separately). The clear broth above the cellulose was gently drawn off the cellulose cultures and discarded; cellobiose cultures were centrifuged, and the supernatant was discarded.

Fifteen milliliters of freshly prepared 5% glutaraldehyde in 0.11 M potassium phosphate (pH 6.1) was added to fix the cells; this buffer was chosen because it had an osmolarity equivalent to that of the medium, as determined by freezing-point depression. After 1 h of fixation, the cellulose or cells were collected on a 0.40-μm-pore membrane filter (Nuclepore) by gentle suction and washed with 1 ml of buffer. The filter was folded in half and sealed in a crimped aluminum foil envelope; windows in the foil allowed infiltration of reagents. The samples were prepared by the technique of Thomas and McMeekin (42) and were viewed with a Cambridge Mark II scanning electron microscope operated at an accelerating voltage of 10 kV.

Nutritional studies. Nutritional requirements were determined in 2XAB broth, with 0.4% cellobiose as the substrate. Growth was measured as the increase in optical density at 600 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. The culture tubes (15-cm roll tubes; Bellco Glass, Inc., Vineland, N.J.) served as the cuvettes. New stoppers were employed because used stoppers were frequently contaminated with residual vitamins.

Determination of molar base ratios. The cells from 200 ml of a late log-phase cellobiose broth culture were collected by centrifugation, and the pellet was frozen on dry ice-ethanol. The pellet was resuspended in 12.5 ml of sucrose buffer (37) and incubated for 10 min with 20 mg of lysozyme (Sigma Chemical Co., St. Louis, Mo.). Nuclease-free pronase (Calbiochem, La Jolla, Calif.) and sarcosate (M Chemical Co., Gardena, Calif.) were added to concentrations of 0.05 and 10 mg/ml, respectively, and the suspension was incubated for an additional 10 min. DNA was purified from the lysed cells by the method of Price et al. (37) through the second precipitation step and redissolution in phosphate buffer.

The buoyant density of each DNA sample was determined in triplicate by centrifugation in a cesium chloride gradient; DNA from *Micrococcus lysodeikticus* served as an internal standard. The molar base ratios were calculated by the formula of Schildkraut et al. (39).

RESULTS

Cellulolytic bacteria were isolated from rat cecal contents by selective isolation in PMC agar or by enrichment in PMC broth followed by selective isolation; the isolation procedures used and the numbers of cellulolytic bacteria determined have been described (34). In the ceca of 16 of 21 rats, the predominant culturable cellulolytic bacteria were gram-negative rods; the predominant organisms isolated from the ceca of the remaining rats were gram-positive diplococci.

Characteristics of the cellulolytic rod-shaped bacteria. Forty-seven strains of cellulolytic rods were isolated from the ceca of 17 rats. The rods formed a homogeneous group based on morphology, growth characteristics in PMC agar and cellobiose agar media, and the ability to ferment selected substrates, with the exception that four strains (from different rats) did not ferment glucose. Representative strains of the glucose fermenters and nonfermenters were selected for further characterization, and three rumen strains of *B. succinogenes*, S85, A3c, and REH9-1, were included in certain experiments for comparison.

The rat isolates were slim rods approximately 0.4 μm wide and 0.8 to 2.0 μm long (Fig. 1), frequently in pairs, especially when growing rapidly. Cells of REH9-1 (Fig. 2A) were similar in size and shape to those of the rat isolates. Cells of S85 were generally coccoid but asymmetrical (Fig. 2B; 32), although short, plump rods were occasionally observed. Where the cells of S85 grew closely together on PMC, they appeared to conform to the shape of neighboring cells (Fig. 2C). The spaces between the cells presumably resulted from shrinkage during

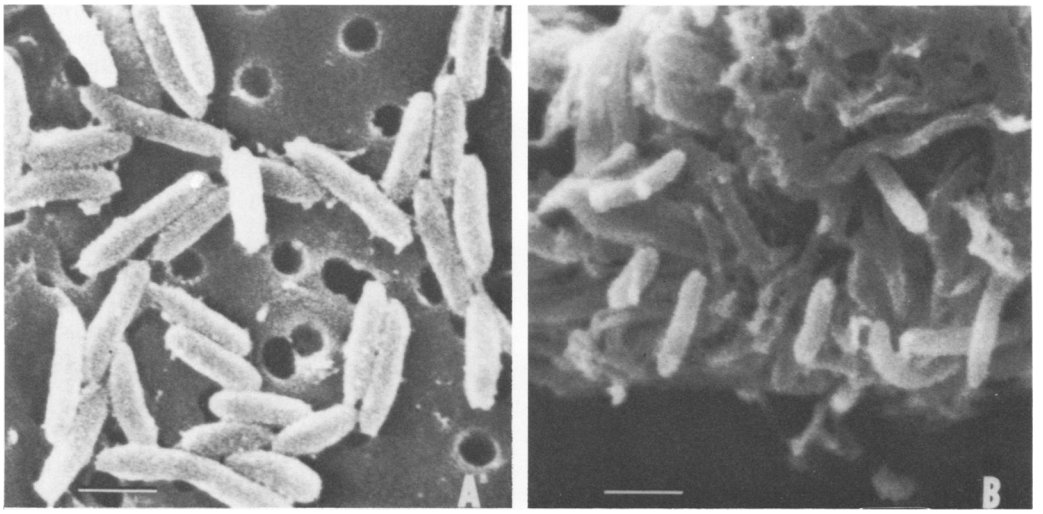

FIG. 1. Scanning electron micrographs of *Bacteroides* sp. strain NR9 grown on cellobiose (A) and cellulose (B). Bar, 1 μm.

preparation for electron microscopy; this may have been shrinkage of the capsule that S85 forms (15).

In all strains, microscopic examination of cellobiose-grown cultures entering stationary phase showed increasing numbers of degeneration forms, which ranged from spheroplasts (11) to bulgy or elongated cells. The degeneration forms of S85 were larger and more irregular than those of the rat isolates. For all strains, the appearance of degeneration forms as growth ceased was accompanied by decreases in turbidity and viability. The decrease in turbidity was more rapid for S85 than for the rat isolates and most rapid for REH9-1. Cultures of the rat isolates in PMC broth resumed growth with little lag when transferred after having been stored at 4°C for 5 to 7 days.

During growth of all strains in PMC broth, turbidity was not observed in the broth above the settled cellulose until most of the cellulose had been digested, at which time a thin layer of turbidity was occasionally visible immediately above the settled cellulose. Phase microscopy showed that most of the cells of S85 were attached to cellulose particles, frequently as microcolonies. In PMC cultures of the rat strains and REH9-1, the number of unattached cells visible was about the same as in cultures of S85, but few cells were seen attached to cellulose, i.e., relatively few cells were visible. Scanning electron microscopy revealed that the cells of these strains were also frequently attached to cellulose (Fig. 1B and 2); presumably, the attached cells of the strains other than S85 were difficult to see by phase microscopy because of their thinness and the heterogeneous nature of the cellulose. The cells also may have been partially hidden within eroded areas of the fibers, such as those made by unidentified rumen bacteria observed by transmission electron microscopy (20).

In PY-CF cellobiose agar, colonies of all strains were lenticular in shape and translucent and honey-brown when illuminated by transmitted light; they frequently looked grainy. In cellulose agar roll tubes, the rat isolates and REH9-1 formed circular clearings in the cellulose; colonies were either not visible or were diffuse and became less distinct upon further incubation. The clearings took 5 or more days to become visible. Microscopic examination of material from the center of the clearings showed numerous spheroplasts; rods were found only at the periphery of the clearings, and the number of viable cells, as determined by enumeration in cellulose or PY cellobiose agar, was very low. Thus, it appeared that the cells had somehow migrated through the agar (26) and that the cellulase was not diffusible in agar. When 0.1% cellobiose was added to PMC agar, distinct colonies formed before clearing of the cellulose was detectable, and these colonies remained visible. Although S85 was originally reported to be unable to clear cellulose in agar medium (6), it was able to do so in PMC agar. Clearing by this organism, however, was considerably slower than that by the rat isolates and REH9-1.

Of the potential substrates tested, only cellulose and cellobiose were fermented by all of the rat isolates (Table 1). Glucose was fermented by 43 of the 47 strains and did not inhibit the growth of a nonfermenting strain.

Strains S85 and REH 9-1 fermented cellulose,

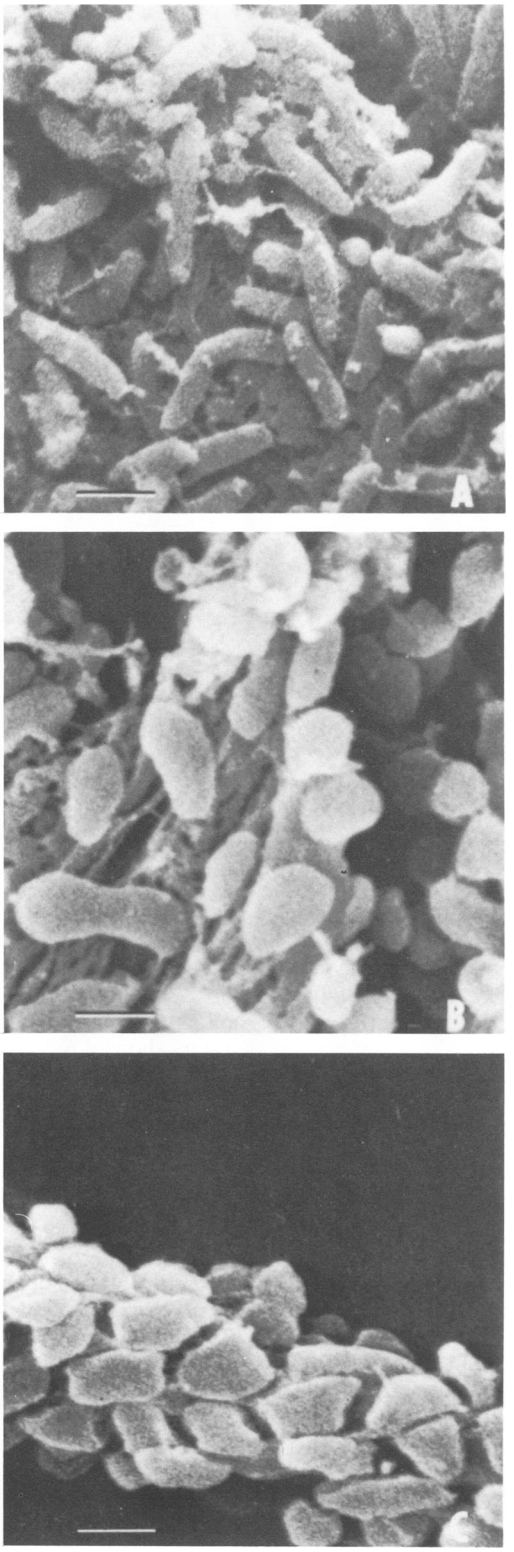

FIG. 2. Scanning electron micrographs of *Bacteroides* sp. strains REH9-1 (A) and S85 (B and C) grown on cellulose. Bar, 1 μm.

cellobiose, and glucose, and S85 also fermented lactose slowly. Neither strain fermented maltose, soluble starch, corn starch, esculin, pectin, or trehalose.

Strains from the cecum and rumen (A3c was not examined) degraded PMC more rapidly than other forms of cellulose, such as Whatman no. 1 filter paper that had not been pebble milled, absorbent cotton (Red Cross brand), or Sigmacell 20 (Sigma). Neither high- nor low-viscosity carboxymethyl cellulose (Sigma) supported growth. Pebble-milled spinach was degraded more rapidly than was PMC, but degradation of the cellulose component was not specifically measured.

During growth on cellulose or cellobiose, the major products formed by the rat isolates were succinic and acetic acids (Table 2). Hydrogen, D- and L-lactic acids, and VFA other than acetic acid were not detected.

Nutritional experiments were designed to assist in developing a simple defined medium and to aid in characterizing the rat isolates. Preliminary experiments showed that strains BCO1 and BCX7 grew well on cellobiose in the basic mineral medium to which had been added the vitamin mix and the VFA mix. The lag phase was often long and variable when minerals were present at standard concentrations; doubling the mineral concentrations resulted in a shorter lag. Thus, the higher concentration (2XAB) was adopted for all media, even though growth in PMC broth had been good with the lower concentration of salts.

Growth of the rat strains was good when the VFA mix was replaced by valeric and 2-methylbutyric acids, but there was no growth without VFA or with either of these two VFA added singly.

Vitamin requirements were determined by deleting pools of vitamins and then adding back the components. Two to three transfers (inoculum, 1 to 4%) in medium deficient in specific vitamins were required to dilute out the residual vitamins from the original inoculum; a washed inoculum was not used because of the fragility of the cells.

Representative data in Table 3 show that *p*-aminobenzoic acid (PABA), cyanocobalamine (B_{12}), and thiamine were required for normal growth of the rat strains. When those vitamins were present, hemin or the other vitamins of the complete mix did not significantly stimulate growth. Substitution of methionine for B_{12} allowed slow growth. These vitamin requirements were confirmed in cellulose broth. No cellulose disappearance was observed after four transfers without PABA or thiamine; when B_{12} was omitted, cellulose disappearance occurred but was considerably slower than in the control with all three vitamins present.

TABLE 1. Characteristics of cellulolytic *Bacteroides* sp. strains isolated from the rat cecum and bovine rumen

Isolation and strain	Morphology	Fermentation					Nutrient requirements					
		Cellulose	Cellobiose	Glucose	Lactose	Others	Straight-chain VFA	Branched-chain VFA	PABA	B_{12}	Thiamine	Biotin
Cecum[a]												
BCO1	Rod	+	+	−	−	−[b]	+	+	+	+	+	−
BCX7	Rod	+	+	+	−	−[b]	+	+	+	+	+	−
Rumen												
REH9-1	Rod	+	+	+	−	−[c]	+[d]	+	+	−	−	+
S85	Coccoid	+	+	+	+ (slow)	−[c]	+[e]	+	−	−	−	+

[a] The isolates did not produce indole, reduce nitrate, or liquify gelatin. (The same results have been reported for S85 [10] and other strains [23].)

[b] The substrates which did not support growth were amygdalin, arabinose, erythritol, esculin, fructose, galactose, glycerol, glycogen, inositol, inulin, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, threonine, trehalose, xylose, soluble starch, corn starch, pectin, and hemicellulose.

[c] See text for the substrates tested.

[d] Nutrient requirements of REH9-1 are from unpublished data.

[e] Nutrient requirements of S85 are from the results of Bryant et al. (8).

To show there were no undiscovered requirements, 12 successive transfers (inoculum, 4%) of BCO1 and BCX7 were made with only the following growth factors in PMC broth: PABA, B_{12}, thiamine, valeric acid, and 2-methylbutyric acid (PBT broth). The same vitamin requirements were found for strain NR9, which was isolated after only three transfers of the original (mixed) culture in PMC broth with cecal extract and the complete vitamin and VFA mixes. From the time of isolation, NR9 was maintained in PBT broth through seven transfers (inoculum, 4%). Since cysteine was present in all media, we cannot rule out a requirement for it.

The mole percent guanine plus cytosine (mol% G+C) of the DNA from three rat isolates was found to be 45.2 to 45.3%; the values for S85, A3c, and REH9-1 were 48.0, 48.8, and 50.7%, respectively (Table 4). Our value for S85 is considerably higher than that previously published (42.8% [38]), which was calculated from buoyant density data by a different formula. When the published buoyant density is converted by the widely accepted formula of Schildkraut et al. (39), a value of 47.0% is obtained, with which our results agree reasonably well. A similar recalculation of the earlier data for strain A3c, however, changes the calculated mol% G+C from 49.1%, which is close to our finding, to 53.7%. Our results were reproducible for three samples each of DNA from S85 (cultures obtained from two sources) and NR9. In addition, the value obtained for *E. coli* K-12 agreed with the accepted value of 51.0% (calculated from a buoyant density of 1.7100 [39]).

TABLE 2. Products of cellulose fermentation by *Bacteroides* sp. strains isolated from the rat cecum and bovine rumen[a]

Acid	Product (mmol/liter)				
	Cecum			Rumen	
	NR9	BC01	BCX7	REH9-1	S85
Succinic	11.7	11.1	10.9	14.0	12.2
Acetic	3.2	3.5	4.4	4.8	3.9
Formic	≤1	<1	<1	<1	<1

[a] Cultures were incubated for 5 days in PMC broth with VFA other than valeric and 2-methylbutyric acids omitted.

We have concluded that the cellulolytic rods isolated from rat ceca are members of *B. succinogenes* based on their morphology, VFA requirements, fermentation of cellulose, production of large amounts of succinate and acetate, and nature of growth in cellulose agar.

Characteristics of the cellulolytic cocci. Cells of the five strains isolated were approximately 0.7 to 0.8 μm in diameter and generally occurred as diplococci, frequently in chains of 4 to 10 cells. Single cells were rare. Scanning electron micrographs (not shown) demonstrated that the morphology of strain BCL1 was similar to that of *R. flavefaciens* C94 (33), although the chains of BCL1 were shorter.

In PY-CF cellobiose agar, colonies appeared similar to those of the *B. succinogenes* strains (above), although slightly darker. Colonies in PMC agar were in the center of clearings in the cellulose and were shaped like multiple disks. Cells were found only in the colony, indicating that they produced a diffusible cellulase.

All strains fermented cellulose and cellobiose; one of four strains tested fermented lactose,

TABLE 3. Vitamin requirements of cellulolytic *Bacteroides* sp. strains isolated from the rat cecum

Supplement				Optical density at 600 nm[a]	
PABA	Thiamine	B_{12}	Methionine	BC01	BCX7
+	+	+	−	1.28	1.15
−	+	+	−	0.13	0.04
+	−	+	−	0.07	0.02
+	+	−	−	0.09	0.03
+	+	−	+	0.57	0.49

[a] Average peak optical density of two cultures after three transfers without the appropriate supplement(s).

melibiose, and raffinose weakly. The remaining substrates tested did not support growth (Table 5). The major products of cellobiose fermentation were succinic and acetic acids; D- and L-lactic acids and VFA other than acetic acid were not detected.

Growth of the cocci was poor in PY-CF cellobiose broth even if supplemented with vitamins and VFA. The highest observed optical density was approximately one-third that for the *B. succinogenes* strains (ca. 0.45 to 0.49 versus 1.4). Substituting rumen fluid for cecal extract did not help, and when neither was present growth ceased after several transfers. The growth factor(s) required by the cocci was not identified.

The strains of cellulolytic cocci isolated from rat ceca have been identified as *R. flavefaciens* based on their morphology, fermentation of cellulose to succinate and acetate, and nature of growth in cellulose agar.

DISCUSSION

The predominant cellulolytic organisms isolated from the ceca of most rats were gram-negative rods identified as strains of *B. succinogenes*. This identification was based on the organism's fermentation of cellulose to succinate and acetate, VFA requirements, morphology, and unique nature of clearing in cellulose agar (25, 26). The first two of these characteristics are consistent in all members of the species. The morphology of *B. succinogenes* strains ranges from slim rods, such as the rat isolates and REH9-1 (16, 17, 25) through broader rods (6, 41) to the coccoid cells of S85 and other isolates (6, 32). The ability to form clearings in cellulose agar seems to correlate with morphology; the slim rods are most active. However, the constitution of the medium affects the expression of this characteristic, since excessive agar concentration can prevent the formation of detectable clearings (34). Thus, observations made in different laboratories are not directly comparable. For example, it is possible that strain BL2, reportedly unable to clear cellulose in agar (41), could do so in PMC agar.

Most of the rat isolates and some rumen isolates (17, 41) (REH9-1) ferment only cellulose, cellobiose, and glucose among the substrates tested. A few rat isolates do not ferment glucose, and some rumen isolates ferment one or more additional substrates (6, 25). *B. succinogenes* strains seem to have a tendency to lose fermentative capabilities; strain BL2 lost the ability to ferment (albeit weakly) maltose, starch, and xylan (41), and S85 may have lost the ability to ferment pectin (6, 10, 18). It is possible that the rat isolates which did not ferment glucose when tested had lost that ability (perhaps the expression of a glucose permease) during repeated transfers in cellulose medium.

The primary phenotypic difference between the rat and rumen isolates is in their vitamin requirements. The three rat isolates examined required PABA, B_{12}, and thiamine, whereas rumen strains require biotin and, in some cases, PABA (8, 40).

The rat and rumen isolates also differ significantly in mol% G+C (45 versus 48 to 51%, respectively). The 6% spread of mol% G+C values found for the species *B. succinogenes* is larger than in most other species, although *Pseudomonas stutzeri*, for example, has a similar range (9). In light of the general phenotypic similarity of the rat isolates to the rumen isolates, the difference in their mol% G+C probably does not justify their exclusion from the species *B. succinogenes*; such exclusion would require the formation of a new species comprising only the rat isolates. To date, not enough strains have been examined to determine whether the rumen organisms comprise two or more groups with discontinuous mol% G+C values (Table 4) or one group with a continuum of values extending over a range of 3% or more. Indeed, there may also be strains, from the same or other environments, with mol% G+C values

TABLE 4. DNA base composition of cellulolytic *Bacteroides* sp. strains isolated from the rat cecum and bovine rumen and of *E. coli* K-12

Strain	Habitat	Buoyant density[a]	mol% G+C
NR9	Cecum	1.7043 ± 0.0003	45.2
BCX7	Cecum	1.7043 ± 0.0001	45.2
BC01	Cecum	1.7044 ± 0.0003	45.3
S85	Rumen	1.7071 ± 0.0002	48.0
A3c	Rumen	1.7078 ± 0.0002	48.8
REH9-1	Rumen	1.7097 ± 0.0001	50.7
K-12	Intestine	1.7097 ± 0.0001	50.7

[a] Mean ± standard deviation. Three samples each of DNA were isolated from NR9 and S85 and one each from the other strains; three determinations were made for each sample.

falling between those known for the rat and rumen strains.

The information presently available suggests that the rat isolates belong in the species *B. succinogenes*, although they appear to form a distinct subgroup that may later be found to deserve subspecies or species status. Clarification of the taxonomy of this species would require examination not only of more strains from the rumen and rat cecum but also of strains from different sources, such as the cecum of ruminants. Cellulolytic *Bacteroides* spp. isolated from the horse intestine (16) and from the rumen-like stomach of the langur monkey (2) were not characterized in sufficient detail to allow comparison with the strains discussed here. Those strains are apparently not available for further study, but improved methods of isolating cellulolytic *Bacteroides* spp. (34) should simplify the isolation of strains from the guts of various animals. Clearings resembling those made by *B. succinogenes* have been observed in cellulose agar inoculated with gut contents of the eland, kongoni, zebu, or camel, but the responsible organisms were not isolated (28). The *Bacteroides* sp. isolated from human feces has been well characterized and was not considered to be a member of *B. succinogenes* (3). A technique such as nucleic acid homology (30) or immunological measurement of protein sequence homology (13) would probably reveal more about the relationships among members of the species than would phenotypic comparisons, as these organisms have too few suitable traits for numerical analysis (14).

The other, although less frequent, cellulolytic isolates from rat ceca were cocci identified as strains of *R. flavefaciens* based on their morphology, fermentation of cellulose to succinate and acetate, and nature of growth in cellulose agar. The apparent requirement of these isolates for a rich medium is also reported for some strains of *R. flavefaciens* isolated from the rumen (7, 29) and from the guinea pig cecum (19); the unidentified growth factor requirements may or may not be the same for the various strains. Other rumen strains of *R. flavefaciens* have simple requirements for vitamins and VFA (7, 29, 40). Nucleic acid base composition has not been measured in this species.

The two predominant species of cellulose-degrading bacteria in rat ceca, *B. succinogenes* and *R. flavefaciens*, are also the two most important in the rumen. The other species of cellulolytic bacteria found in the rumen, including *Ruminoccus albus, Butyrivibrio fibrisolvens, Eubacterium cellulosolvens*, and *Clostridium* spp., have not been isolated from the rat cecum.

We are initiating an investigation of the physiology of cellulose degradation by the *B. succino-*

TABLE 5. Characteristics of *Ruminococcus* sp. strains isolated from the rat cecum and the bovine rumen

Isolation and strain	Morphology	Fermentation[a]											Others[b]	Complex nutrient requirement	Fermentation product
		Cellulose	Cellobiose	Lactose	Melibiose	Raffinose	Glucose	Fructose	Maltose	Salicin	Arabinose	Sucrose	Mannose	Amygdalin	
Cecum (4 strains)	Diplococcus, chains	+	+	¼ W	¼ W	¼ W	–	–	–	–	–	–	–	–	–	+	Succinate, acetate[c]
Rumen[d]																	
R. flavefaciens	Diplococcus, chains	+	V	V	–	–	V	V	V	V	V	–	–	–	–	V[e]	Succinate, acetate
R. albus	Diplococcus	+	V	+	–	–	V	V	–	V	–	V	V	V	–	V[e]	Ethanol, acetate

[a] ¼ W, Weak fermentation by one of four strains; V, variable.

[b] The other substrates tested (four rat strains) were erythritol, esculin, galactose, glycerol, glycogen, inositol, inulin, mannitol, melezitose, rhamnose, ribose, sorbitol, soluble starch, threonine, trehalose, and xylose.

[c] H_2 and formate not determined.

[d] From Anaerobe Laboratory Manual (23).

[e] From Bryant and Robinson (7) and Jarvis and Annison (29).

genes strains isolated from the rat cecum. It is believed that the cells of this species must contact the cellulose substrate and that, in agar medium, the cells must migrate through the agar to achieve that contact (25, 26). The cellulase system of *B. succinogenes* has been suggested to be cell bound (32), and recent work with S85 supports that hypothesis (21).

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